



UM01531.ST25.txt
SEQUENCE LISTING

<110> Hale, Calvin C
Price, Elmer M

<120> LARGE SCALE EXPRESSION AND PURIFICATION OF RECOMBINANT PROTEINS

<130> UMO 1531.1

<140> US 09/901,419

<141> 2001-07-09

<160> 5

<170> PatentIn version 3.1

<210> 1

<211> 4087

<212> DNA

<213> Bos taurus

<220>

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<222> (268)..(3180)

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<221> sig_peptide

<222> (268)..(363)

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<221> misc_feature

<222> (3178)..()

<223> A Poly (H) affinity tag comprising 6 His residues have been inserted at the C-Terminus end of the coding region of the protein

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cagagtctcg agatgcgacg ctacaatctg cagttttcac tagcttccca gtaggttggg      240
acagttggaa ctctgccatt gcccagc atg ctg cag ttc agt ctg tca ccc acc      294
                               Met Leu Gln Phe Ser Leu Ser Pro Thr
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ttg tcg atg gga ttt cac gtg ata gcc atg gtg gct ctc ttg ttt tcc      342
Leu Ser Met Gly Phe His Val Ile Ala Met Val Ala Leu Leu Phe Ser
10                               15                               20                               25

cat gtg gac cat ata agt gct gag aca gaa atg gaa gga gaa ggc aac      390
His Val Asp His Ile Ser Ala Glu Thr Glu Met Glu Gly Glu Gly Asn
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gag act ggc gag tgt act ggc tcc tat tac tgt aag aag ggg gtg att      438
Glu Thr Gly Glu Cys Thr Gly Ser Tyr Tyr Cys Lys Lys Gly Val Ile
                               45                               50                               55

tta ccc att tgg gag ccc cag gac cct tcc ttt gga gac aaa att gct      486
Leu Pro Ile Trp Glu Pro Gln Asp Pro Ser Phe Gly Asp Lys Ile Ala
                               60                               65                               70

aga gcg act gtg tat ttt gtg gcc atg gtc tac atg ttt ctt gga gtc      534
Arg Ala Thr Val Tyr Phe Val Ala Met Val Tyr Met Phe Leu Gly Val
                               75                               80                               85

tca atc att gct gac cgg ttc atg tcc tct ata gaa gtc atc acg tct      582
Ser Ile Ile Ala Asp Arg Phe Met Ser Ser Ile Glu Val Ile Thr Ser
90                               95                               100                               105

caa gag aaa gaa atc acc ata aag aaa ccc aat gga gag acc acc aag      630
Gln Glu Lys Glu Ile Thr Ile Lys Lys Pro Asn Gly Glu Thr Thr Lys
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aca act gtg agg atc tgg aat gag aca gtg tcc aac ctg acc ttg atg      678
Thr Thr Val Arg Ile Trp Asn Glu Thr Val Ser Asn Leu Thr Leu Met
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gcc ctg ggg tct tca gct cca gag att ctc ctt tca gta atc gag gtg      726
Ala Leu Gly Ser Ser Ala Pro Glu Ile Leu Leu Ser Val Ile Glu Val
                               140                               145                               150

tgt ggc cat aac ttc act gca gga gac ctt ggc cct agc acc atc gtg      774
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gac agg agg ctt ctg ttt tac aag tat gtc tac aag agg tat cgg gct Asp Arg Arg Leu Leu Phe Tyr Lys Tyr Val Tyr Lys Arg Tyr Arg Ala 250 255 260 265	1062
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caa ggg aca tat cag tgt ctg gag aac tgt ggc aca gta gcc ctg acc Gln Gly Thr Tyr Gln Cys Leu Glu Asn Cys Gly Thr Val Ala Leu Thr 410 415 420 425	1542

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780										785					790					
tac	tgg	aac	ggc	tgg	gcg	tgt	ttc	atc	gtc	tcc	atc	ctc	atg	atc	ggc	2694				
Tyr	Trp	Asn	Gly	Trp	Ala	Cys	Phe	Ile	Val	Ser	Ile	Leu	Met	Ile	Gly					
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Gln	Tyr	Ala	Asp	Ala	Ser	Ile	Gly	Asn	Val	Thr	Gly	Ser	Asn	Ala	Val					
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Asn	Val	Phe	Leu	Gly	Ile	Gly	Val	Ala	Trp	Ser	Ile	Ala	Ala	Ile	Tyr					
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Phe																	
970																	
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<213> Bos taurus

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<221> misc_feature

<222> (3178)..()

<223> A Poly (H) affinity tag comprising 6 His residues have been inser

ted at the C-Terminus end of the coding region of the protein

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Glu Thr Glu Met Glu Gly Glu Gly Asn Glu Thr Gly Glu Cys Thr Gly
35      40      45

Ser Tyr Tyr Cys Lys Lys Gly Val Ile Leu Pro Ile Trp Glu Pro Gln
50      55      60

Asp Pro Ser Phe Gly Asp Lys Ile Ala Arg Ala Thr Val Tyr Phe Val
65      70      75      80

Ala Met Val Tyr Met Phe Leu Gly Val Ser Ile Ile Ala Asp Arg Phe
85      90      95

Met Ser Ser Ile Glu Val Ile Thr Ser Gln Glu Lys Glu Ile Thr Ile
100     105     110

Lys Lys Pro Asn Gly Glu Thr Thr Lys Thr Thr Val Arg Ile Trp Asn
115     120     125

Glu Thr Val Ser Asn Leu Thr Leu Met Ala Leu Gly Ser Ser Ala Pro
130     135     140

Glu Ile Leu Leu Ser Val Ile Glu Val Cys Gly His Asn Phe Thr Ala
145     150     155     160

Gly Asp Leu Gly Pro Ser Thr Ile Val Gly Ser Ala Ala Phe Asn Met
165     170     175

Phe Ile Ile Ile Ala Leu Cys Val Tyr Val Val Pro Asp Gly Glu Thr
180     185     190

Arg Lys Ile Lys His Leu Arg Val Phe Phe Val Thr Ala Ala Trp Ser
195     200     205

Ile Phe Ala Tyr Thr Trp Leu Tyr Ile Ile Leu Ser Val Ser Ser Pro
210     215     220

Gly Val Val Glu Val Trp Glu Gly Leu Leu Thr Phe Phe Phe Phe Pro
225     230     235     240

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Ile Cys Val Val Phe Ala Trp Val Ala Asp Arg Arg Leu Leu Phe Tyr
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Lys Tyr Val Tyr Lys Arg Tyr Arg Ala Gly Lys Gln Arg Gly Met Ile
260 265 270

Ile Glu His Glu Gly Asp Arg Pro Ser Ser Lys Thr Glu Ile Glu Met
275 280 285

Asp Gly Lys Val Val Asn Ser His Val Asp Ser Phe Leu Asp Gly Ala
290 295 300

Leu Val Leu Glu Val Asp Glu Arg Asp Gln Asp Asp Glu Glu Ala Arg
305 310 315 320

Arg Glu Met Ala Arg Ile Leu Lys Glu Leu Lys Gln Lys His Pro Glu
325 330 335

Lys Glu Ile Glu Gln Leu Ile Glu Leu Ala Asn Tyr Gln Val Leu Ser
340 345 350

Gln Gln Gln Lys Ser Arg Ala Phe Tyr Arg Ile Gln Ala Thr Arg Leu
355 360 365

Met Thr Gly Ala Gly Asn Ile Leu Lys Arg His Ala Ala Asp Gln Ala
370 375 380

Arg Lys Ala Val Ser Met His Glu Val Asn Thr Glu Val Ala Glu Asn
385 390 395 400

Asp Pro Val Ser Lys Ile Phe Phe Glu Gln Gly Thr Tyr Gln Cys Leu
405 410 415

Glu Asn Cys Gly Thr Val Ala Leu Thr Ile Ile Arg Arg Gly Gly Asp
420 425 430

Leu Thr Asn Thr Val Phe Val Asp Phe Arg Thr Glu Asp Gly Thr Ala
435 440 445

Asn Ala Gly Ser Asp Tyr Glu Phe Thr Glu Gly Thr Val Val Phe Lys
450 455 460

Pro Gly Glu Thr Gln Lys Glu Ile Arg Val Gly Ile Ile Asp Asp Asp
465 470 475 480

Ile Phe Glu Glu Asp Glu Asn Phe Leu Val His Leu Ser Asn Val Lys
485 490 495

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      500                      505                      510
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Ser Thr Leu Ala Cys Leu Gly Ser Pro Ser Thr Ala Thr Val Thr Ile
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.
Phe Asp Asp Asp His Ala Gly Ile Phe Thr Phe Glu Glu Pro Val Thr
      530                      535                      540
:
His Val Ser Glu Ser Ile Gly Ile Met Glu Val Lys Val Leu Arg Thr
      545                      550                      555                      560
.
Ser Gly Ala Arg Gly Asn Val Ile Val Pro Tyr Lys Thr Ile Glu Gly
      565                      570                      575
.
Thr Ala Arg Gly Gly Gly Glu Asp Phe Glu Asp Thr Cys Gly Glu Leu
      580                      585                      590
.
Glu Phe Gln Asn Asp Glu Ile Val Lys Thr Ile Ser Val Lys Val Ile
      595                      600                      605
.
Asp Asp Glu Glu Tyr Glu Lys Asn Lys Thr Phe Phe Leu Glu Ile Gly
      610                      615                      620
.
Glu Pro Arg Leu Val Glu Met Ser Glu Lys Lys Ala Leu Leu Leu Asn
      625                      630                      635                      640
.
Glu Leu Gly Gly Phe Thr Ile Thr Gly Lys Tyr Leu Tyr Gly Gln Pro
      645                      650                      655
.
Val Phe Arg Lys Val His Ala Arg Glu His Pro Leu Pro Ser Thr Ile
      660                      665                      670
.
Ile Thr Ile Ala Asp Glu Tyr Asp Asp Lys Gln Pro Leu Thr Ser Lys
      675                      680                      685
.
Glu Glu Glu Glu Arg Arg Ile Ala Glu Met Gly Arg Pro Ile Leu Gly
      690                      695                      700
.
Glu His Thr Arg Leu Glu Val Ile Ile Glu Glu Ser Tyr Glu Phe Lys
      705                      710                      715                      720
.
Ser Thr Val Asp Lys Leu Ile Lys Lys Thr Asn Leu Ala Leu Val Val
      725                      730                      735
.
Gly Thr Asn Ser Trp Arg Glu Gln Phe Ile Glu Ala Ile Thr Val Ser
      740                      745                      750

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Ala Gly Glu Asp Asp Asp Asp Asp Glu Cys Gly Glu Glu Lys Leu Pro
755 760 765

Ser Cys Phe Asp Tyr Val Met His Phe Leu Thr Val Phe Trp Lys Val
770 775 780

Leu Phe Ala Phe Val Pro Pro Thr Glu Tyr Trp Asn Gly Trp Ala Cys
785 790 795 800

Phe Ile Val Ser Ile Leu Met Ile Gly Leu Leu Thr Ala Phe Ile Gly
805 810 815

Asp Leu Ala Ser His Phe Ala Cys Thr Ile Ala Leu Lys Asp Ser Val
820 825 830

Thr Ala Val Val Phe Val Ala Leu Gly Thr Ser Val Pro Asp Thr Phe
835 840 845

Ala Ser Lys Val Ala Ala Thr Gln Asp Gln Tyr Ala Asp Ala Ser Ile
850 855 860

Gly Asn Val Thr Gly Ser Asn Ala Val Asn Val Phe Leu Gly Ile Gly
865 870 875 880

Val Ala Trp Ser Ile Ala Ala Ile Tyr His Ala Ala Asn Gly Glu Gln
885 890 895

Phe Lys Val Ser Pro Gly Thr Leu Ala Phe Ser Val Thr Leu Phe Thr
900 905 910

Ile Phe Ala Phe Ile Asn Val Gly Val Leu Leu Tyr Arg Arg Arg Pro
915 920 925

Glu Ile Gly Gly Glu Leu Gly Gly Pro Arg Thr Ala Lys Leu Leu Thr
930 935 940

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Leu Glu Ala Tyr Cys His Ile Lys Gly Phe
965 970

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Glu Thr Glu Met Glu Gly Glu Gly Asn Glu Thr Gly Glu Cys Thr Gly
 35 40 45

Ser Tyr Tyr Cys Lys Lys Gly Val Ile Leu Pro Ile Trp Glu Pro Gln
 50 55 60

Asp Pro Ser Phe Gly Asp Lys Ile Ala Arg Ala Thr Val Tyr Phe Val
 65 70 75 80

Ala Met Val Tyr Met Phe Leu Gly Val Ser Ile Ile Ala Asp Arg Phe
 85 90 95

Met Ser Ser Ile Glu Val Ile Thr Ser Gln Glu Lys Glu Ile Thr Ile
 100 105 110

Lys Lys Pro Asn Gly Glu Thr Thr Lys Thr Thr Val Arg Ile Trp Asn
 115 120 125

Glu Thr Val Ser Asn Leu Thr Leu Met Ala Leu Gly Ser Ser Ala Pro
 130 135 140

Glu Ile Leu Leu Ser Val Ile Glu Val Cys Gly His Asn Phe Thr Ala
 145 150 155 160

Gly Asp Leu Gly Pro Ser Thr Ile Val Gly Ser Ala Ala Phe Asn Met
 165 170 175

Phe Ile Ile Ile Ala Leu Cys Val Tyr Val Val Pro Asp Gly Glu Thr
 180 185 190

Arg Lys Ile Lys His Leu Arg Val Phe Phe Val Thr Ala Ala Trp Ser
 195 200 205

Ile Phe Ala Tyr Thr Trp Leu Tyr Ile Ile Leu Ser Val Ser Ser Pro
 210 215 220

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Gly Val Val Glu Val Trp Glu Gly Leu Leu Thr Phe Phe Phe Phe Pro
225 230 235 240

Ile Cys Val Val Phe Ala Trp Val Ala Asp Arg Arg Leu Leu Phe Tyr
245 250 255

Lys Tyr Val Tyr Lys Arg Tyr Arg Ala Gly Lys Gln Arg Gly Met Ile
260 265 270

Ile Glu His Glu Gly Asp Arg Pro Ser Ser Lys Thr Glu Ile Glu Met
275 280 285

Asp Gly Lys Val Val Asn Ser His Val Asp Ser Phe Leu Asp Gly Ala
290 295 300

Leu Val Leu Glu Val Asp Glu Arg Asp Gln Asp Asp Glu Glu Ala Arg
305 310 315 320

Arg Glu Met Ala Arg Ile Leu Lys Glu Leu Lys Gln Lys His Pro Glu
325 330 335

Lys Glu Ile Glu Gln Leu Ile Glu Leu Ala Asn Tyr Gln Val Leu Ser
340 345 350

Gln Gln Gln Lys Ser Arg Ala Phe Tyr Arg Ile Gln Ala Thr Arg Leu
355 360 365

Met Thr Gly Ala Gly Asn Ile Leu Lys Arg His Ala Ala Asp Gln Ala
370 375 380

Arg Lys Ala Val Ser Met His Glu Val Asn Thr Glu Val Ala Glu Asn
385 390 395 400

Asp Pro Val Ser Lys Ile Phe Phe Glu Gln Gly Thr Tyr Gln Cys Leu
405 410 415

Glu Asn Cys Gly Thr Val Ala Leu Thr Ile Ile Arg Arg Gly Gly Asp
420 425 430

Leu Thr Asn Thr Val Phe Val Asp Phe Arg Thr Glu Asp Gly Thr Ala
435 440 445

Asn Ala Gly Ser Asp Tyr Glu Phe Thr Glu Gly Thr Val Val Phe Lys
450 455 460

Pro Gly Glu Thr Gln Lys Glu Ile Arg Val Gly Ile Ile Asp Asp Asp
465 470 475 480

Ile Phe Glu Glu Asp Glu Asn Phe Leu Val His Leu Ser Asn Val Lys
485 490 495

Val Ser Leu Glu Ala Ser Glu Asp Gly Ile Leu Glu Ala Ser His Val
500 505 510

Ser Thr Leu Ala Cys Leu Gly Ser Pro Ser Thr Ala Thr Val Thr Ile
515 520 525

Phe Asp Asp Asp His Ala Gly Ile Phe Thr Phe Glu Glu Pro Val Thr
530 535 540

His Val Ser Glu Ser Ile Gly Ile Met Glu Val Lys Val Leu Arg Thr
545 550 555 560

Ser Gly Ala Arg Gly Asn Val Ile Val Pro Tyr Lys Thr Ile Glu Gly
565 570 575

Thr Ala Arg Gly Gly Gly Glu Asp Phe Glu Asp Thr Cys Gly Glu Leu
580 585 590

Glu Phe Gln Asn Asp Glu Ile Val Lys Thr Ile Ser Val Lys Val Ile
595 600 605

Asp Asp Glu Glu Tyr Glu Lys Asn Lys Thr Phe Phe Leu Glu Ile Gly
610 615 620

Glu Pro Arg Leu Val Glu Met Ser Glu Lys Lys Ala Leu Leu Leu Asn
625 630 635 640

Glu Leu Gly Gly Phe Thr Ile Thr Gly Lys Tyr Leu Tyr Gly Gln Pro
645 650 655

Val Phe Arg Lys Val His Ala Arg Glu His Pro Leu Pro Ser Thr Ile
660 665 670

Ile Thr Ile Ala Asp Glu Tyr Asp Asp Lys Gln Pro Leu Thr Ser Lys
675 680 685

Glu Glu Glu Glu Arg Arg Ile Ala Glu Met Gly Arg Pro Ile Leu Gly
690 695 700

Glu His Thr Arg Leu Glu Val Ile Ile Glu Glu Ser Tyr Glu Phe Lys
705 710 715 720

Ser Thr Val Asp Lys Leu Ile Lys Lys Thr Asn Leu Ala Leu Val Val
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730

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Gly Thr Asn Ser Trp Arg Glu Gln Phe Ile Glu Ala Ile Thr Val Ser
 740 745 750

Ala Gly Glu Asp Asp Asp Asp Asp Glu Cys Gly Glu Glu Lys Leu Pro
 755 760 765

Ser Cys Phe Asp Tyr Val Met His Phe Leu Thr Val Phe Trp Lys Val
 770 775 780

Leu Phe Ala Phe Val Pro Pro Thr Glu Tyr Trp Asn Gly Trp Ala Cys
 785 790 795 800

Phe Ile Val Ser Ile Leu Met Ile Gly Leu Leu Thr Ala Phe Ile Gly
 805 810 815

Asp Leu Ala Ser His Phe Ala Cys Thr Ile Ala Leu Lys Asp Ser Val
 820 825 830

Thr Ala Val Val Phe Val Ala Leu Gly Thr Ser Val Pro Asp Thr Phe
 835 840 845

Ala Ser Lys Val Ala Ala Thr Gln Asp Gln Tyr Ala Asp Ala Ser Ile
 850 855 860

Gly Asn Val Thr Gly Ser Asn Ala Val Asn Val Phe Leu Gly Ile Gly
 865 870 875 880

Val Ala Trp Ser Ile Ala Ala Ile Tyr His Ala Ala Asn Gly Glu Gln
 885 890 895

Phe Lys Val Ser Pro Gly Thr Leu Ala Phe Ser Val Thr Leu Phe Thr
 900 905 910

Ile Phe Ala Phe Ile Asn Val Gly Val Leu Leu Tyr Arg Arg Arg Pro
 915 920 925

Glu Ile Gly Gly Glu Leu Gly Gly Pro Arg Thr Ala Lys Leu Leu Thr
 930 935 940

Ser Cys Leu Phe Val Leu Leu Trp Leu Leu Tyr Ile Phe Phe Ser Ser
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